1-7-2007

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A. H. Tart
Methodist Hospital Research Institute and Department of Pathology, Houston USA

Mark J. Walker
University of Wollongong, mwalker@uow.edu.au

J. M. Musser
Methodist Hospital Research Institute and Department of Pathology, Houston USA

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Keywords

Group A Streptococcus, human pathogens, infections, therapeutic drugs

Disciplines

Life Sciences | Physical Sciences and Mathematics | Social and Behavioral Sciences

Publication Details

Tart, AH, Walker, MJ and Musser, JM, New understanding of the group A streptococcus pathogenesis cycle, Trends in Microbiology 15, 318-325, 2007. Copyright 2007 Elsevier

New understanding of the group A *Streptococcus* pathogenesis cycle

Anne H. Tart[1], Mark J. Walker[2], and James M. Musser[1*]

[1]Center for Molecular and Translational Human Infectious Diseases Research, The Methodist Hospital Research Institute and Department of Pathology, The Methodist Hospital, Houston, Texas, USA; [2]School of Biological Sciences, University of Wollongong, Wollongong, New South Wales, Australia.

*Corresponding author:

James M. Musser, M.D., Ph.D.

Email: (JMMusser@tmh.tmc.edu)

Abstract

Group A *Streptococcus* (GAS) has long been recognized as a human pathogen causing an exceptionally broad range of infections. However, despite intense research, the molecular mechanisms of GAS disease remain unclear. Recently, many important discoveries have been made that shed light on GAS pathogenesis and open exciting new avenues for future research. Advances in genome sequencing, microarray technology and proteomic analysis in combination with the development of more suitable animal models have dramatically increased the amount of data regarding the mechanisms of GAS pathogenesis. The information gained from these studies will translate into the identification of improved diagnostics and new targets for novel therapeutic drugs and vaccines.

Epidemiologic versatility of group A *Streptococcus*

The Gram-positive bacterium *Streptococcus pyogenes* (group A *Streptococcus*, GAS) causes a range of human infections such as the clinically uncomplicated conditions pharyngitis ("strep throat") and impetigo, whilst also causing severe invasive diseases including necrotizing fasciitis (NF, "flesh-eating disease") and streptococcal toxic shock syndrome (STSS). In addition, the post-infection immune sequelae glomerulonephritis and acute rheumatic fever may occur. To cause such a wide spectrum of disease, GAS must be able to adapt to the diverse physiological conditions encountered in the human host. For example, the pathogen must overcome the first line of physical and immune defense in saliva to successfully colonize the oropharynx. Moreover, GAS must disseminate from the initial site of infection to cause invasive disease, a process which requires the bacterium to break out of the local site of infection and survive in the blood. To accomplish each of these transitions, GAS has evolved sophisticated strategies and complex regulatory mechanisms that allow it to thwart host defenses and successfully colonize, thrive and/or persist in the host. Many proven and putative virulence factors made by GAS contribute to host-pathogen interactions (Fig. 1). In this review, we discuss recent advances in the understanding of GAS pathogenesis and focus on the role of a variety of streptococcal virulence factors in the establishment and dissemination of disease. While the mechanistic basis of the regulation of many of these virulence factors is extremely important, a complete treatment of this topic is beyond the scope of this review.

Survival of GAS in human saliva

The oropharynx is the primary site of GAS entry into the human host. In this environment, GAS encounters saliva, which contains components of the innate and the acquired immune system including antimicrobial peptides that limit the growth of pathogenic bacteria. It has been known since the 1940s that saliva containing high levels of GAS plays a role in the person-to-person spread of this pathogen[1-3]. Recent *in vitro* analysis of the interaction between human saliva and GAS reveals that the microorganism has evolved a mechanism that allows it to persist in saliva at relatively high viable cell numbers for prolonged periods of time[4]. This phenomenon is specific for saliva, as GAS grown in either limited or defined liquid media did not remain viable for long time periods. It was also demonstrated that serotype M1 strains of GAS reach higher concentrations in human saliva than strains of other serotypes. Considering that M1 strains are a leading cause of streptococcal pharyngitis[5], it may be that the relatively high density achieved in saliva by certain GAS serotypes enhances their likelihood of being transmitted to a new host.

Shelburne *et al.*[6] discovered that a two-component regulatory system of previously unknown function mediates the persistence of GAS in human saliva. This two-component system, designated *sptR/S* for saliva persistence, directly or indirectly controls expression of about 8% of the GAS genome in exponential growth phase and a striking 20% of the genome during stationary growth phase[6]. Of the genes controlled by *sptR/S*, genes involved in carbohydrate metabolism are most abundantly represented. One of the genes discovered to aid GAS survival in saliva is *malE,* which encodes a highly conserved maltodextrin-binding protein present in a broad range of GAS strains[6,7]. Several lines of evidence suggest that MalE is required for successful interaction of GAS with human saliva. Firstly, Shelburne *et al.*[7] showed that an isogenic *malE* mutant is impaired in its ability to grow and persist in saliva *in vitro* compared to the parental GAS strain. These data imply that MalE may play a crucial role in the GAS-saliva interaction by contributing to nutrient acquisition. Since saliva contains only low levels of glucose, GAS depends on additional carbon sources for growth. One of the components found in saliva is amylase, which degrades ingested starch to smaller chains of carbohydrates. MalE may enable GAS to utilize these particular carbohydrates as additional energy sources. In addition, MalE may be essential for the earliest stages of GAS colonization as evidenced by high *malE* transcript levels early in the GAS-saliva interaction[7]. Thus, human saliva may represent an important reservoir for GAS, which is critical for both persistence and transmission of the pathogen.

Pharyngeal disease

Although GAS is able to cause serious invasive disease, pharyngitis is by far the most common infection. For example, ~15 million cases of streptococcal pharyngitis occur annually in the US alone, resulting in an estimated 2 billion dollars of direct health care costs[8]. However, despite decades of research, our knowledge of the precise molecular events mediating GAS pharyngitis remains rudimentary. Recently, three strategies have been used to expand our understanding regarding the pathogenesis of this infection. One line of productive research that has yielded new information about GAS-host interactions has used mouse nasal-associated lymphoid tissue (NALT)[9-11]. Use of NALT and a bioluminescent-tagged GAS strain has suggested that M cells in NALT transport GAS across the epithelial cell layer, perhaps facilitating invasion of deeper tissues[9]. NALT also has been used to study immune responses to GAS[10].

A second line of investigation has used gene transcript analysis of GAS in primary throat swabs obtained from patients with pharyngitis. Virtaneva *et al.*[12] used real time reverse-transcription PCR (RT-PCR) to study transcripts of 17 GAS genes in throat swab specimens obtained from 18 pediatric patients diagnosed with acute pharyngitis. This method allowed the analysis of GAS gene expression *in vivo,* thereby opening a new avenue of investigation. Genes belonging to two component regulatory systems, regulatory genes, and genes encoding proven and putative virulence factors were studied. Although the level of gene transcripts varied between individuals, several notable trends emerged. For example, *fasC,* a gene encoding a member of a gene regulatory system, was highly expressed during acute pharyngitis. The *fasBCA(X)* regulates expression of fibronectin-binding proteins, superoxide dismutase, streptolysin S-associated genes, and streptokinase[13]. Another report suggested that the Fas regulon is upregulated under amino acid starvation, which may be encountered during initial contact of the pathogen with the skin and throat or at a site of high cell densities in a nidus of infection[14]. Virtaneva *et al.*[12] also showed that the *mga* (multi-gene activator) and *perR* regulatory genes were highly expressed during acute pharyngitis. Mga controls expression of genes encoding important GAS virulence factors including *emm* (M protein), *mac* (inhibitor of phagocytosis), and *sic* (streptococcal inhibitor of complement), all of which have been implicated in either adhesion to host cells or evasion from host defenses. Less is known about *perR*, although there is evidence that it plays a role in iron homeostasis and the oxidative stress response. It has been shown to be upregulated *in vitro* under iron-limiting conditions[15], which GAS may encounter within the host during infection where most iron is sequestered by iron-binding proteins and only minute amounts of free iron are available[16]. Of the 7 extracellular proteins examined, 4 (*scpA* [C5a peptidase], *bspA* [cell surface protein], *edin* [homolog to *Staphylococcus aureus* EDIN toxin],and *sda* [DNase]) were upregulated during acute pharyngitis as determined by real-time RT-PCR[12].

A third line of research has used a cynomolgus macaque model of pharyngitis [12, 17-19]. Due to high human host specificity of GAS, rodent models are inadequate in their representation of pharyngitis pathogenesis because they neither precisely mimic human GAS disease nor the host response against GAS infection. Virtaneva *et al.*[17] used expression microarray analysis to study longitudinal changes of GAS gene expression in

20 cynomolgus macaques experimentally infected with serotype M1 strain MGAS5005. This strain is genetically representative of M1 GAS strains which are currently responsible for a large percentage of pharyngitis and invasive infections[20]. The monkeys developed acute pharyngitis that was indistinguishable from human infection. This observation was noteworthy as other non-human primate models such as baboons[21] and rhesus macaques[22,23] can be successfully colonized with GAS in the upper respiratory tract but do not develop pharyngitis that mimics human disease. The discovery that cynomolgus macaques develop acute streptococcal pharyngitis that is a phenocopy of human disease has resulted in this animal model being treated as the gold standard for GAS pharyngitis studies.

Many pathogenic bacteria, including GAS, produce extracellular DNase, and it has been speculated that GAS DNase functions as a virulence factor. The contribution of a phage-encoded DNase to the pathogenesis of infection caused by contemporary M1 strains has an interesting history that warrants brief review. In 1989, Stevens and colleagues[24] observed that contemporary episodes of severe invasive GAS infections in the Rocky Mountain Region of the United States were associated with scarlet fever toxin A (SpeA), long known to be encoded by a bacteriophage. This crucial observation was confirmed by analyzing clonal diversity and genetic relationships among 108 strains of GAS recovered from patients throughout the USA[25]. Importantly, Southern blot analysis indicated that there were at least two distinct genotypes of serotype M1 strains, those that were *speA*-positive, and those that lacked this gene and presumably the bacteriophage encoding it. Further analysis revealed that contemporary serotype M1 isolates contained a distinct *speA* allele[26]. Cleary *et al.*[27] confirmed the association of the *speA* gene with severe serotype M1 GAS infections and extended the concept that two distinct subclones of serotype M1 existed. Importantly, the analysis revealed that relative to *speA*-negative strains, *speA*-positive strains contained approximately 70 kb of additional DNA. Subsequently, mouse virulence studies showed that *speA*-positive organisms were significantly more virulent[28]. A detailed genetic dissection of 126 GAS strains demonstrated considerable genetic diversity existed among M1 strains[20]. The analysis showed that a distinct virulent serotype M1 subclone had emerged recently and spread intercontinentally. It was then discovered that some contemporary M1 isolates contain a gene encoding a DNase that is absent in the genome of strain SF370[29]. The DNase gene was linked to a phage gene, providing supportive evidence that contemporary serotype M1 strains were genetically distinct. Sumby *et al.*[18] sequenced the genome of serotype M1 strain MGAS5005 and found that it had genes encoding three distinct DNases (*spd3*, *sdaD2* and *spd*), two of which are bacteriophage-encoded. Seven isogenic mutant strains were constructed and used to study the role of DNases in host-pathogen interactions *in vivo*[18]. Importantly, it was found that DNase production is required for normal progression of both pharyngitis and invasive infections. As part of the innate immune response to bacterial infection, neutrophils kill bacteria by entanglement in neutrophil extracellular traps, which consist of DNA and chromatin, and subsequent destruction of the captured pathogen[30]. Sumby *et al.*[18] demonstrated that GAS DNase activity is crucial in assisting GAS to avoid destruction by neutrophils, which was confirmed by other investigators[31].

Two additional recent publications have closed the loop on certain aspects of genetic differentiation of contemporary and old serotype M1 strains[32,33]. The occurrence of prophages encoding DNases in contemporary M1 isolates was confirmed[32]. In addition, Sumby *et al.*[33] sequenced the genome of a contemporary M1 strain and discovered that this *speA*-positive genotype originally described in 1991 had a 36-kb recombination region involving genes encoding several GAS toxins, including streptolysin O (SLO) and NAD^+-glycohydrolase (NADase). Importantly, this recombination event is associated with significantly increased production of these two toxins. Sumby *et al.*[33] used the genome sequence information, DNA-DNA microarray, high-throughput single nucleotide polymorphism analysis, and expression microarray analysis to reconstruct the molecular evolutionary events that resulted in the genesis of the abundant serotype M1 clone now responsible for much human disease worldwide.

Vascular leakage and survival of GAS in plasma and blood

When GAS causes superficial infections, it is exposed to human plasma at sites of inflammation as a consequence of vascular leakage, which is mediated in part by the interaction of M protein and fibrinogen with the β_2-integrin adhesion molecule on the surface of neutrophils (PMNs). This complex results in a massive inflammatory response which involves the release of heparin binding protein (HBP) and induces vascular leakage[34]. As augmented vascular permeability is one of the underlying pathophysiological mechanism in shock, the crosslinking of the M protein and fibrinogen with β_2-integrin may play an important role in the establishment of STSS. Plasma is a rich medium that supports bacterial growth, however it also contains many immune system components which GAS must elude to survive, including opsonizing antibodies and complement. Many plasma protein-GAS protein interactions interfere with the proper function of host defenses[5,35]. Moreover, GAS expresses surface proteins that have a high affinity for several human plasma proteins such as albumin, fibrinogen, α_2-macroglobulin, IgG, and plasminogen[5,35-38], which suggests that the pathogen has evolved mechanisms to capture and use host proteins for enhanced survival *in vivo*.

To examine the effect of plasma on GAS protein expression, Johansson *et al.*[39] compared the proteomes of serotype M1 strain AP1 cultured in laboratory medium or in human plasma. Expression of 39 protein spots representing 24 unique GAS proteins, was significantly increased in cells cultured in human plasma. Interestingly, multiple spots for secreted extracellular proteins C5a peptidase and M1 were present in the proteome of plasma-grown bacteria, but were absent in the proteome of strain AP1 grown in laboratory medium. Concurrent with these data, a truncated variant of M1 protein lacking the N-terminal 13 amino acids of the mature, full-length protein was identified, a finding indicative of post-translational modification of this key virulence factor in response to plasma exposure[39].

We have only limited knowledge of changes in protein expression in GAS cultured in blood compared to laboratory media. However, Graham *et al.*[40] have demonstrated that GAS undergoes a rapid and extensive remodeling of its transcriptome when grown in human blood *ex vivo*. Within 30 min after initial contact of GAS with whole blood, transcripts of 716 genes (representing 37% of the GAS genome) were up-regulated whereas 425 transcripts (22% of the genome) were down-regulated. Transcripts of genes belonging to functional categories expected to be important for growth adaptation in blood including *de novo* synthesis of macromolecular precursors, carbohydrate metabolism, membrane transport, and transcriptional regulation were increased. Expression of several virulence genes was up-regulated, including the multigene activator *mga* and its regulated transcripts *emm1, sic*, streptolysin, and *has* (capsular polysaccharide)[40]. Evidence for the up-regulation of capsule expression of GAS in blood *in vivo* was provided by Gryllos *et al.*[41] who showed that there is a temporal relationship between capsule gene expression and proliferation of GAS in the blood of infected mice.

Transition from local to systemic infection

Each year GAS causes an estimated 700 million cases of mild non-invasive infections such as pharyngitis and impetigo worldwide. Approximately 700,000 of these develop into severe invasive disease[42]. The exact mechanism mediating the switch from a localized to a systemic infection remains to be elucidated. One factor that is thought to contribute to this process is the binding of GAS to human plasminogen and its subsequent activation to the broad-spectrum protease plasmin[43-47]. It was hypothesized that plasminogen is supplied by vascular leakage at the site of infection. There is evidence that GAS assembles a trimolecular complex consisting of fibrinogen, plasminogen, and streptokinase, which is associated with a propensity for invasive disease[48,49]. Recent studies using a humanized transgenic mouse model confirmed the important role for human plasminogen in the dissemination of GAS *in vivo*[37,45] and lead to the proposal that GAS may subvert human plasminogen for use as a virulence factor[36,45]. Interestingly, GAS also produces the cysteine protease SpeB, which has been shown to directly degrade the trimolecular complex[48]. Differential regulation of *speB* expression seems to play a role in the transitioning from localized to invasive disease. The *speB* gene is found in over 99% of GAS isolates and is highly conserved, and production of SpeB has been shown to be important in the establishment of localized GAS infections[37,50,51]. Kansal *et al.*[52] observed that SpeB levels vary greatly in both clonally related and unrelated strains and that expression of SpeB and human invasive disease severity are inversely related in M1T1 GAS isolates. In addition, passage of *speB* expressing GAS strains in mice resulted in a downregulation of *speB* concomitantly with the upregulation of M protein expression[52]. Moreover, Cole *et al.*[37] discovered that there is a subpopulation of GAS in localized infections that can lose SpeB activity. This ultimately results in an accumulation of plasmin activity on the GAS cell surface and equips this particular subpopulation with enhanced invasive potential. Ultimately, this may result in the transition of GAS from the local site of infection to the blood and promotes dissemination to other parts of the host.

To gain additional information about the GAS genes expressed during skin and soft tissue infection, Graham *et al.*[53] analyzed the transcriptome of GAS in mouse soft tissue infection. The transcriptome of a serotype M1 strain was studied, and array datasets were verified by quantitative real-time RT-PCR and *in situ* immunohistochemistry. The results demonstrated that coordinated expression of GAS virulence factors is directed toward overcoming innate host defenses, resulting in severe cellular damage. In addition, several classes of genes were found to be highly expressed *in vivo*, including oxidative stress genes, virulence genes, genes related to complex carbohydrate utilization, and several two-component transcriptional regulators. This study was the first global analysis of the GAS transcriptome in invasive infection and produced many new avenues for basic and translational research.

Severe invasive disease

Severe invasive GAS infections have re-emerged on a global level since the mid-1980s. These infections can progress rapidly and are associated with exceedingly high rates of morbidity and mortality[54]. Many strains causing necrotizing fasciitis, an especially severe and destructive infection, are serotype M1 and M3 strains[54-56], which suggests that particular GAS strains have an increased propensity to cause invasive disease.

Sumby *et al.*[57] described two distinct transcriptome profiles of GAS that were linked to the type of disease, i.e. pharyngitis or invasive infection. The pharyngeal transcriptome profile (PTP) and the invasive transcriptome profile (ITP) differ significantly in expression of approximately 10% of the genome, including genes of several proven and putative virulence factors. Strains of the PTP were able to transition to the ITP phenotype during invasive infections, but strains of the ITP phenotype did not convert to the PTP phenotype under the conditions tested. Strains of the ITP phenotype were more resistant than PTP organisms to phagocytosis and killing by human neutrophils. To discover the genetic changes underlying the two distinct transcriptome profiles, complete genome re-sequencing of a mouse-derived ITP strain was conducted[57]. The genome of the ITP derivative organism differed from the PTP precursor strain by only a 7-bp frameshift mutation located in the *covS* gene, which encodes the sensor kinase of the CovR/S two-component regulatory system. CovR/S directly or indirectly controls approximately 15% of the GAS transcriptome[58]. The frameshift mutation truncates CovS, resulting in the de-repression of many genes encoding proven virulence factors, including *speA* (streptococcal pyrogenic exotoxin A), *sagA* (streptolysin S), *mac, ska* (streptokinase), and *spd3.* Consistent with these findings, Engleberg *et al.*[59] reported that spontaneous mutations in CovR/S result in increased virulence in murine skin and soft tissue infections. Thus, the transcriptome profile and the virulence character of GAS are intimately linked to the allelic state of the genes encoding the CovR/S two-component system.

Several lines of evidence have recently implicated a GAS extracellular protease (termed ScpC or SpyCEP) that cleaves interleukin-8 (IL-8) in invasive disease[60-62]. IL-8 is a chemoattractant that promotes recruitment of neutrophils to the site of infection by promoting their migration out of the bloodstream. Thus, the ability of GAS to impair the activity of IL-8 during infection may enhance its survival during infection and aid in its dissemination within the host. SpyCEP specifically cleaves the carboxy-terminus of IL-8, resulting in inactivation of the chemoattractant property of this molecule. This enzyme also cleaves and inactivates mouse chemokines known as KC and MIP-2[62]. Hidalgo-Grass *et al.*[62] reported that inactivation of the gene encoding SpyCEP impairs GAS clearance from infected mouse tissue and is associated with decreased soft tissue infection.

There is also evidence that *mtsR*, a gene encoding a metal transport regulator, may play a role in deep-muscle infections[63-65]. Using comparative genome re-sequencing, Beres *et al.*[65] discovered that strains containing a naturally occurring mutation in *mtsR*, which yields a severely truncated MtsR protein, are significantly underrepresented among GAS isolates recovered from necrotizing fasciitis cases. These results suggest that MtsR is necessary for the full virulence potential of GAS in invasive disease. Expression microarray analysis found that this truncation mutation is linked to significantly altered transcript levels of multiple genes and operons involved in metal homeostasis and response to oxidative stress[65], which perhaps renders the pathogen more vulnerable to the host immune response.

The molecular processes contributing to tissue destruction in GAS necrotizing fasciitis and myonecrosis remain poorly understood. Recent studies have provided important evidence that streptolysin O-induced platelet/neutrophil complexes play a role in development of ischemic necrosis of tissue[66]. In related work, Bryant *et al.*[67] showed that an early step in the pathogenesis of GAS myonecrosis may involve binding of the pathogen to vimentin, a molecule that is up-regulated after skeletal-muscle injury.

Asymptomatic carriage

GAS is exclusively a human pathogen and therefore must survive in a human reservoir. Many individuals carry GAS asymptomatically in the upper respiratory tract and other anatomic sites. Over the years there have been several hypotheses to explain this phenomenon. In principle, asymptomatic carriage of GAS could arise as a consequence of mutations in the pathogen that down-regulate virulence, a productive immune response by the host that constrains pathogen proliferation, internalization into host cells, or some combination thereof. Relatively little work has been done to study the genetic relationships between strains causing disease episodes and those of the same M protein serotype that are recovered from asymptomatic carriers. However, this issue can now be studied at the genome-wide level. To address this issue, Beres *et al.*[68] used comparative genome re-sequencing to show that several serotype M3 strains from asymptomatic throat carriers contained deletion mutations in the pleiotrophic virulence regulatory gene *mga* and the *emm* gene. The occurrence of these mutations in the carrier isolates but not in disease-causing organisms implicates them in down-regulating virulence. Consistent with this idea, carrier strains were significantly less virulent, as assessed by intraperitoneal injection into mice[68]. Thus, these data support the hypothesis that asymptomatic carriage may arise as a consequence of mutations that down-regulate virulence.

While GAS has long been regarded as an extracellular pathogen, there has been evidence that it can invade non-phagocytic cells[69-72]. This observation has led to conjecture that internalization of GAS by host cells participates in the carrier state[5,70,73]. GAS internalization may allow the pathogen to evade certain host defenses and protect it from killing by antibiotics such as penicillin, as this antibiotic does not enter epithelial cells[70]. Consistent with this idea, Sela *et al.*[70] observed that strains escaping antibiotic killing had significantly higher internalization efficiencies than strains that were successfully eliminated by antibiotic therapy. Other investigators have reported that genes encoding proteins that are linked to internalization, such as those encoding fibronectin-binding proteins F2, SfbI, and PfbpI, are significantly more prevalent among persisting GAS strains recovered from asymptomatic carriers[5,73]. However, Brandt *et al.*[74] found no evidence for an increased prevalence of the *prtF1* gene among GAS isolates recovered from pharyngitis patients who had antibiotic treatment failure. Hence, the literature bearing on this issue remains unclear and additional work is required to elucidate the role of host-cell internalization in asymptomatic carriage and other aspects GAS biology and pathogenesis.

Asymptomatic carriage of GAS also may have a role in some invasive infections. Medina and colleagues observed that the intracellular survival of GAS in neutrophils may result in increased bacterial virulence[75]. Using a mouse model, it was shown that ingested GAS is transported by neutrophils to distant parts of the body[75]. This is a notable finding as it may explain the cases of necrotizing fasciitis occurring in the absence of an entry wound or trauma adjacent to the site of GAS infection.

Concluding remarks

This review has highlighted some recent advances in our understanding of GAS pathogenesis and its interaction with the human host at various sites of infection. Many of the important findings have been made possible by contemporary genome sequencing, microarray technology and proteome analysis. Combined use of these technologies permits a global interrogation of molecular events that occur during host-pathogen interactions. Their use has revealed that GAS responds rapidly and in a regulated manner to the distinct environments encountered in the host during the establishment and dissemination of disease (Fig. 2). In the future, these approaches will be useful in identifying molecular markers specific for each phase of the disease process. This is especially important for severe invasive GAS diseases, which progress rapidly and are frequently diagnosed too late due to the current lack of appropriate diagnostics.

Acknowledgements

We thank I. Sitkiewicz, P. Sumby, and K. Stockbauer for helpful criticism of the manuscript. We apologize to investigators whose work we could not cite due to space limitations.

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Box 1: Outstanding questions:

What are the molecular markers in the initial stages of severe invasive diseases such as streptococcal toxic shock syndrome and necrotizing fasciitis?

What is the molecular mechanism responsible for severe invasive GAS infections?

What host factors are involved in the development of, as well as the susceptibility to, severe invasive disease?

What is/are the environmental signal(s) that cause the transcriptional switch from non-invasive to severe invasive streptococcal disease?

Figure Legends

Figure 1: **GAS virulence factors interact with the host at multiple levels.** GAS has an arsenal of virulence factors at its disposal that allow it to successfully colonize and thrive in the host. At the cell and tissue level, these factors contribute to the pathogenicity of GAS by mediating adherence to host cells, by promoting internalization and invasion, and by evading phagocytosis. At the organism level, the virulence factors are involved in facilitating dissemination throughout the host and can induce systemic toxicity. Importantly, many of the proven streptococcal virulence factors function at multiple stages of infection.

Figure 2: Group A *Streptococcus* modifies the transcription of genes belonging to diverse functional groups during infection. GAS causes many different human infections, reflecting its ability to adapt to diverse physiological conditions. Several recent studies have investigated GAS transcriptome remodeling in pharyngitis[17]] and invasive soft-tissue infection[53], and during growth in saliva[6] and blood[40].

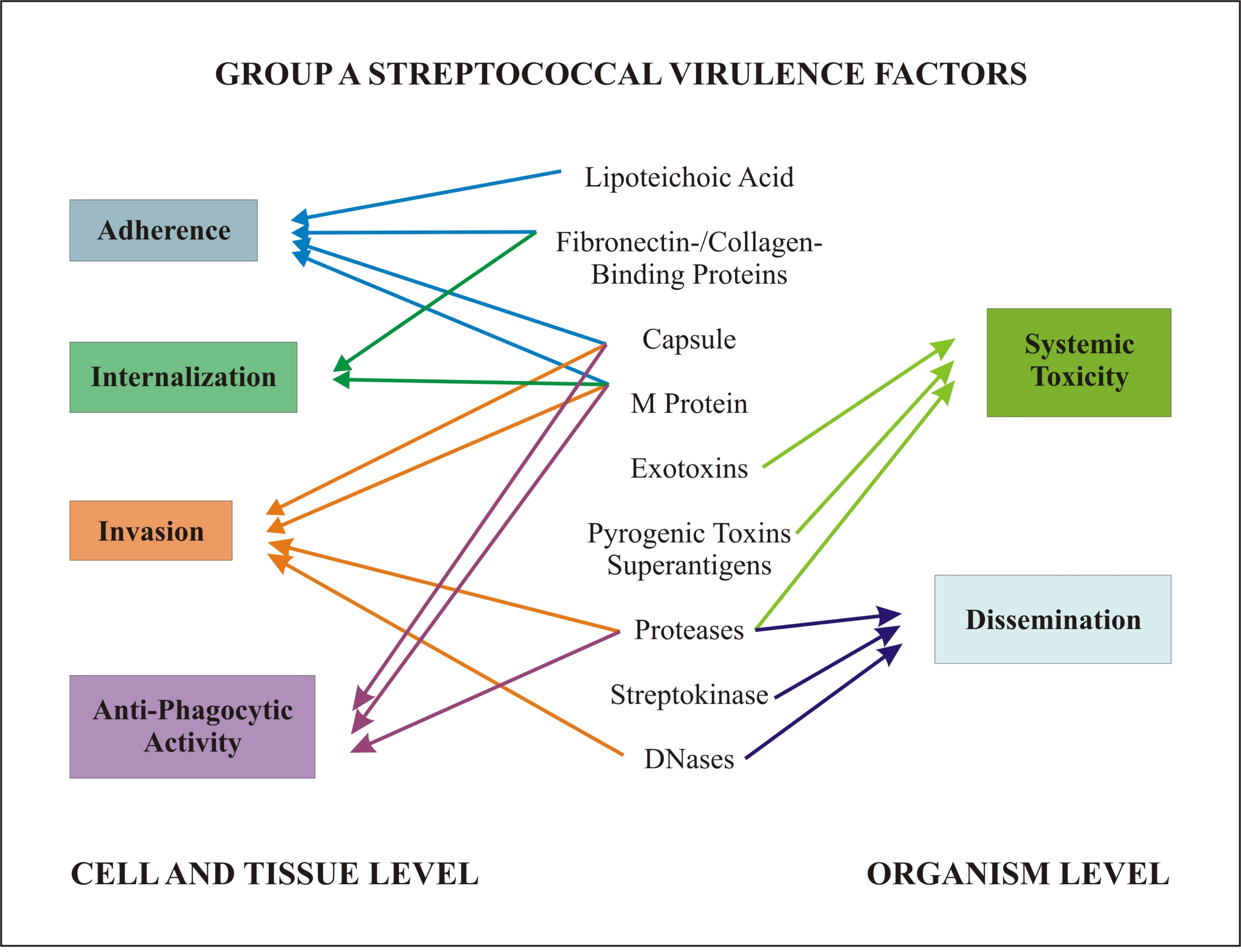

Figure 1: GAS virulence factors interact with the host at multiple levels

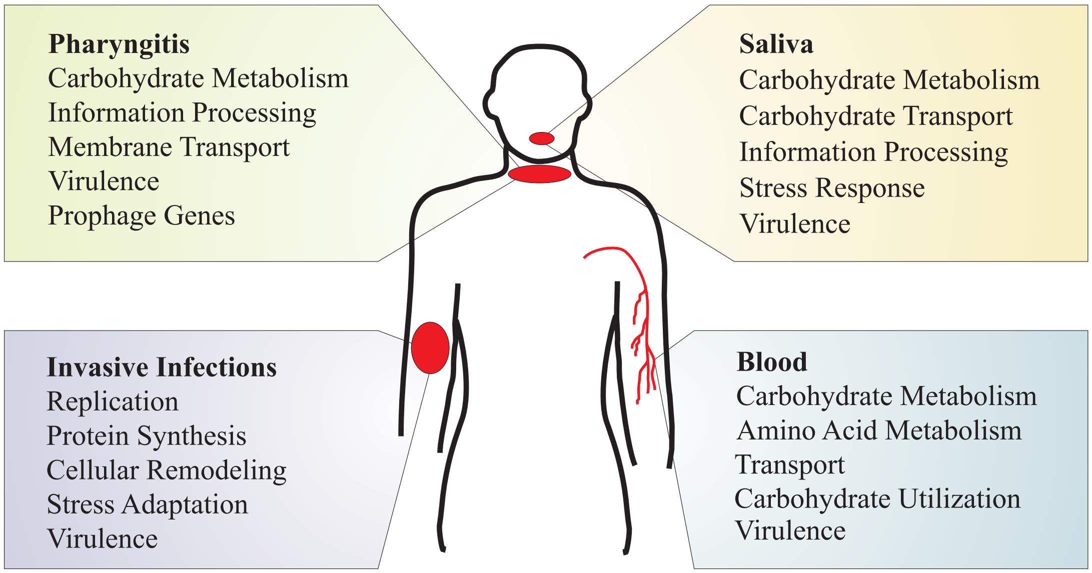

Figure 2: Group A *Streptococcus* Modifies transcription of genes belonging to diverse functional categories during infection.